**Technical report**

**Project Title: Reducing soybean cyst nematode with brown mustard and winter camelina**

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**Introduction**

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe), is an obligatory endo-parasitic nematode distributed worldwide and one of the major yields suppressing pathogens of soybean in the United States, including North Dakota (Allen et al., 2017; Wrather and Koenning, 2009). In North Dakota, since its first detection in 2003 in Richland County (Bradley et al., 2004), SCN has been spreading rapidly in the southeastern counties and confirmed in at least 19 counties in 2017 (Tylka and Marett, 2017).

In a previous study funded by the ND Soybean Council the preceding and interseeding of cover crops into standing soybean [*Glycine max* (L.) Merr.] was studied. The study was established at Prosper and Casselton, ND in 2018. Preceding cover crops before soybean did not establish well since the growing season started too late to allow them for enough growth before soybean was planted. However, the interseeded crops established very well. Results indicated brown mustard (*Brassica juncea* cv. Kodiak) and winter camelina (*Camelina sativa* cv. Joelle) performed the best when interseeded at V6 stage of soybean. Soybean cyst nematode populations were highly variable in the plots at the beginning of the season with counts from 200 to 9,000 eggs/ 100 cm3 of soil.

The research goal of this proposal was to manage SCN populations with brown mustard and winter camelina planted after wheat in the fall or interseeded into soybean in the same season.

**Research objectives**

1. to determine the effect of interseeded brown mustard and winter camelina at V6 stage of soybean on SCN-population
2. to determine if termination of interseeded brown mustard has an effect on SCN-population while avoiding competition with soybean.
3. to determine the effect of brown mustard and winter camelina planted right after wheat harvest on SCN population in susceptible and resistance soybean following wheat.
4. to determine if there is an interaction between resistant and susceptible cultivars and cover crops.

**Methodology**

***Experiment 1: Effect of interseeding winter camelina and brown mustard into standing soybean on SCN populations***

The research was conducted at two sites in Prosper and Casselton, ND sites infested with SCN. The experimental design was a randomized complete block design (RCBD) with a split-plot arrangement with four replicates. The main plot was the soybean cultivar (susceptible or resistant) the subplots were winter camelina cv. Joelle and brown mustard cv. Kodiak interseeded at V6 stage and two check plots with only soybean and one check plot with no soybean or cover crop.

The SCN-resistant cultivar was P10T91 and the SCN-susceptible cultivar was 9OY80 RR.

Three treatments were interseeded with brown mustard at V6, one plot was terminated at R4 stage of soybean (9 August 2019) (Mustard V6 +T), one plot was not terminated (Mustard V6 no T) and in the other soybean was removed in on 8 July (Mustard V6 Soy removed). Soybean main plots were planted approximately on 29 May in Prosper and 4 June in Casselton due to wet soil conditions. Row spacing was 22 inches with a targeted established plant population of 175,000 plants/acre. Two treatments of camelina were interseeded at V6. In one treatment camelina was kept until the end of the season (Camelina V6) and in the other soybean was removed on July 8 (Camelina V6 Soy removed). Since camelina is a winter crop and stays in rosette stage termination as in mustard was not done. This to see if the presence of the soybean, is what is driving SCN reproduction and compare if SCN populations were different in soybean alone or with interseeded cover crop. The treatment with cover crop only (no soybean) was established to see if SCN initial populations decline if soybean is not present.

***Experiment 2: Effect of fall-seeded cover crops on SCN populations in soybean.***

Cover crops were planted on August 23 in Prosper, ND and on August 30 in Crookston, MN after wheat harvest. Winter camelina cv. Joelle and brown mustard cv. Kodiak were planted on 8 ft wide by 25 ft long plots, with four replicates in a RCB design with a split-plot arrangement.

In 2019, susceptible and resistant soybean cultivars of soybean were planted at 22” and 30” row spacings in Crookston (20 May 2019) and Prosper (29 May 2019), respectively. The SCN-resistant cultivars used were P10T91 and AGO3X7, and SCN-susceptible cultivars 9OY80 RR and AGO3X8 at Prosper and Crookston, respectively.

***Soybean cyst nematode sampling* *and other measurements***: Soil samples were collected from each field plot before planting and after harvesting the cover crops and the soybean to determine the initial nematode population densities and final nematode population densities. Cysts were extracted from each sample and then crushed to release the eggs. The eggs were collected and counted under a microscope. The nematode population was expressed as the number of eggs in 100 cm3 of soil. Reproductive factors were calculated by dividing the final population density by the initial population density to evaluate the effect of each cover crop on SCN population reduction. The SCN HG type at Prosper and Casselton was determined as HG type 0. Crookston SCN population was not typified. Soybean grain yield and plant height, cover crops dry matter biomass yield, and soybean crude protein and oil content were also measured.

***Statistical Analysis:*** Statistical analysis was conducted using standard procedure for a randomized complete block design with a split-plot arrangement. Different cover crops treatments were considered fixed effects. Analysis of variance and mean comparison was conducted using SAS procedures; mean separation was performed using LSD at *P* < 0.05.

**Results**

***Experiment 1:*** Interseeded cover crops did not reduced soybean seed yield in Prosper (Table 1). In Casselton, the mustard interseeded at V6 and without mid-season termination reduced soybean yield by 11 bu/acre in the SCN-susceptible soybean and by 7 bu/acre in the SCN-resistant soybean cultivar (Table 2). Soybean plant height was not different among treatments (Table 1,2). Cover crop biomass yield across treatments was higher in the SCN-resistant cultivar. Camelina had the lowest biomass yield when soybean was not removed at both locations (Table1,2). Upon removal of soybean both mustard and camelina increased biomass yield. Winter camelina is a winter crop, staying as a rosette while mustard bolted and flowered producing much more biomass than camelina.

The SCN initial populations in the spring were not different among treatments but they were about twice as high in Prosper compared with Casselton (Table 3). The high variability in the distribution of SCN in each experiment, makes it difficult to determine significant differences among treatments. The combined analysis of variance detected significant differences only for the interaction between cultivar and location for initial SCN population and cultivar for fall-SCN population. Treatments, cultivar, and the interaction between them were not significant for the reproductive factor (RF) at any location. However, if results are analyzed by location in Prosper the RF was different for cultivar, treatment, and cultivar by treatment. Reproductive factors >1 indicate the final SCN population was greater than the initial population.

The SCN-resistant cultivar had a much lower RF than the SCN-susceptible cultivar across all treatments, both in Prosper and Casselton (Table 3). In addition, treatments in which soybean was removed had lower RF. The RF in the interseeded cover crops treatments was the same as the SCN-susceptible soybean check with no cover crop. This indicates that in the presence of a SCN-susceptible cultivar, cover crops were not able to reduce the SCN reproduction. The SCN-resistant cultivar clearly reduced SCN reproduction across all treatments. In Prosper, camelina and brown mustard interseeded at V6 did have a lower RF in the SCN-resistant cultivar compared with the soybean control. It appears interseeded cover crops aid the SCN-resistant cultivar to keep the SCN-population low and this could be a management practice to reduce the risk of SCN overcoming the resistance of the current cultivars.

Crude protein (CP) and oil content were different among treatments and cultivars at both locations (Table 4). In general, check treatments of soybean without cover crops had higher CP and lower oil content than treatments with interseeded cover crops. The lowest soybean CP was observed in the treatment with mustard interseeded at V6 stage and not terminated.

**Table 1. Soybean grain yield and plant height and cover crops dry matter biomass yield for the SCN-susceptible and SCN-resistant cultivars and two cover crops (CC) planted interseeded at V6 stage in Prosper, ND.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Susceptible | | | Resistant | | |
| Cover crop treatment | Soybean yield | Plant height | CC biomass yield | Soybean yield | Plant height | CC biomass yield |
|  | Bu/acre | inches | lbs/acre | Bu/ acre | inches | lbs/acre |
| Camelina V6 | 31.2 | 21.5 | 1947c | 30.7 | 21.0 | 2484c |
| Mustard V6 + T | 30.5 | 21.0 | . | 34.1 | 21.8 | . |
| Mustard V6 no T | 30.5 | 20.2 | 9495b | 24.4 | 19.8 | 9688b |
| CamelinaV6 + Soy removed | . | . | 4916bc | . | . | 5433bc |
| Mustard V6 + Soy removed | . | . | 19183a | . | . | 23126a |
| Check 1-soybean no CC | 34.3 | 21.2 | . | 35.7 | 21.2 | . |
| Check 2- no soybean no CC | 32.3 | 20.8 | . | 34.6 | 21.5 | . |
| LSD (0.05) | NS | NS | 5268 | NS | NS | 5268 |
| Mean cultivar | 31.7 | 21.0a | 8886b | 31.9 | 21.0a | 10696a |

Different small case letters indicate significant difference (*P* ≤ 0.05) between susceptible and resistant cultivar for the same parameter evaluated. T = termination of mustard on August 9; Removal of soybean plants on July 8.

**Table 2. Soybean grain yield and plant height and cover crops dry matter biomass yield for the SCN-susceptible and SCN-resistant cultivars and two cover crops (CC) planted interseeded at V6 stage in Casselton, ND.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Susceptible | | | Resistant | | |
| Cover crop treatment | Soybean yield | Plant height | CC biomass yield | Soybean yield | Plant height | CC biomass yield |
|  | Bu/acre | inches | lbs/acre | Bu/acre | inches | lbs/acre |
| Camelina V6 | 21.0a | 17.0 | 2175d | 17.2a | 14.8 | 1730d |
| Mustard V6 + T | 18.3a | 15.5 | . | 20.7a | 13.8 | . |
| Mustard V6 no T | 9.6b | 14.5 | 6875b | 14.5b | 12.8 | 8197b |
| CamelinaV6 + Soy removed | . | . | 4627bc | . | . | 4435c |
| Mustard V6 + Soy removed | . | . | 13366a | . | . | 15241a |
| Check 1-soybean no CC | 18.9a | 15.8 | . | 17.2a | 14.0 | . |
| Check 2- no soybean no CC | 21.7a | 15.2 | . | 19.1a | 13.8 | . |
| LSD (0.05) | 4.2 | NS | 2135 | 4.2 | NS | 2135 |
| Mean cultivar | 17.9 | 15.6 | 6761a | 17.6 | 13.8 | 7401a |

Different small case letters indicate significant difference (*P* ≤0.05) between susceptible and resistant cultivar for the same parameter evaluated. T = termination of mustard on August 9; Removal of soybean plants on July 8.

**Table 3. SCN populations and reproductive factor (RF) in spring and fall in soybean in interseeded winter camelina and brown mustard (CC) in V6 stage into standing soybean. SCN-susceptible (S) and SCN-resistant (R) soybean cultivars at Prosper and Casselton, ND.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Prosper | | | |  | Casselton | | | |  |
| Treatment | Spring | Fall-S | Fall-R | RF-S | RF-R | Spring | Fall-S | Fall-R | RF-S | RF-R |
|  | ----SCN eggs/100 cm3---- | | |  |  | --SCN eggs/100 cm3-- | | |  |  |
| Camelina V6 | 4695 | 1740 | 310 | 1.3bc | 0.05c | 725 | 2550 | 535 | 5.5 | 0.49 |
| Mustard V6 +T | 4679 | 2150 | 1080 | 3.2ab | 0.13b | 1170 | 1520 | 590 | 6.0 | 0.45 |
| Mustard V6 no T | 4679 | 5853 | 550 | 4.9a | 0.07c | 1170 | 2010 | 355 | 7.5 | 0.21 |
| Camelina V+Soy-Removed | 4965 | 500 | 1340 | 0.6c | 0.17b | 515 | 1110 | 102 | 18.5 | 1.07 |
| Mustard V6 +Soy- Removed | 4175 | 855 | 3630 | 1.1bc | 0.39a | 650 | 730 | 1105 | 1.1 | 1.53 |
| Check 1- soybean no CC | 3185 | 1820 | 1445 | 5.0a | 0.24a | 990 | 1225 | 600 | 2.2 | 0.33 |
| Check 2 -no soy no CC | 4000 | 1670 | 470 | 0.4c | 0.08c | 630 | 1510 | 1440 | 3.1 | 1.17 |
| Mean |  | 1905 | 1260 | 2.17 | 0.16 |  | 1459 | 784 | 5.61 | 0.97 |
|  | *P<F* | | | | | | | | | |
| *Cultivar* | ***0.005*** | *0.14* | | ***0.0052*** | | *0.11* | *0.06* | | ***0.009*** | |
| *Trt* | *0.782* | *0.51* | | ***0.0007*** | | *0.12* | *0.56* | | *0.390* | |
| *Cultivar x Trt* | *0.182* | *0.08* | | ***0.0006*** | | *0.83* | *0.18* | | *0.340* | |
| *Cultivar x Loc* | ***<0.001*** | *0.79* | | *0.280* | |  |  |  |  |  |
| *Trt x Loc* | *0.825* | *0.519* | | *0.487* | |  |  |  |  |  |

T = mustard plots were terminated in one treatment to avoid competition from overgrowth. Small case letters are to compare means within a same column.

**Table 4. Soybean crude protein (CP) and oil content for the SCN-susceptible and SCN-resistant cultivars interseeded with camelina or brown mustard (CC) at V6 stage in Prosper and Casselton, ND.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Prosper | | | | Casselton | | | |
|  | Susceptible | |  | Resistant | Susceptible | | Resistant | |
| Treatment | CP | Oil | CP | Oil | CP | Oil | CP | Oil |
|  | ----------------------------------------%------------------------------------------ | | | | | | | |
| Camelina V6 | 34.2b | 17.7b | 35.4a | 17.2b | 35.3 | 17.8ª | 34.3 | 17.8a |
| Mustard V6 + T | 34.6a | 18.0a | 35.0a | 17.7ª | 35.6 | 17.8ª | 34.3 | 17.9a |
| Mustard V6 no T | 33.9b | 17.8a | 34.6b | 17.4b | 36.3 | 17.0b | 34.2 | 17.6a |
| Check 1-soybean no CC | 34.5a | 17.6b | 35.2a | 17.2b | 35.7 | 17.7a | 34.3 | 17.9a |
| Check 2- soybean no CC | 34.7a | 17.5b | 35.4a | 17.1b | 35.6 | 17.7a | 34.8 | 17.6a |
| LSD (0.05) | 0.4 | 0.2 | 0.4 | 0.2 | NS | 0.3 | NS | 0.3 |
| Mean cultivar | 34.4a | 17.8a | 35.1a | 17.3b | 35.7a | 17.6a | 34.4b | 17.7a |

Different small case letters indicate significant difference (*P* ≤ 0.05) between SCN-susceptible and SCN-resistant cultivar for the same parameter evaluated. Crude protein and oil content were corrected at 13% moisture.

***Experiment 2:***

Soybean cyst nematode populations had an interesting fluctuation over time. No differences were observed among cover crop treatments or cultivars in the soil collected after the cover crops establishment in the fall of 2018 or in the spring of 2019 at either location (Table 5). The SCN population density increased from spring to fall in the plots planted to the SCN-susceptible cultivar which had significantly greater SCN egg densities than plots planted to the SCN-resistant cultivar at both locations.

The winter camelina treatment had significantly lower SCN-egg densities in plots planted to the SCN-susceptible cultivar compared with the no-cover check plots in Prosper, but not in Crookston. In plots planted to the SCN-resistant cultivar, both cover crops treatments had lower SCN numbers than the no-cover check plots at both locations. This response may indicate that cover crops can aid the SCN-resistant cultivar to keep SCN from reproducing. Even though in plots planted to the SCN-resistant cultivar, SCN population densities increased 2- to 5-fold from spring to fall in Prosper. This is worrisome, indicating that SCN-resistant cultivars on their own may not be capable of stopping SCN reproduction completely, resulting in increased SCN population densities over time. In contrast, in Crookston, the SCN-resistant cultivar was able to suppress SCN reproduction in all treatments. As in Prosper, there was a trend to have a greater SCN suppression in plots that had the winter camelina or mustard cover crop treatments in the SCN-resistant cultivar.

In plots planted to the SCN-susceptible cultivar cover crops did not differ from the soybean control. Cover crops planted in the fall aided the SCN-resistant cultivar to suppress SCN population growth. In a greenhouse study, it was shown both winter camelina and brown mustard suppressed SCN by 60% and 51%, respectively (Acharya et al., 2019), which also supports the results and trends observed in these experiments. More research is needed to confirm these results, but given the great variability in SCN distribution in the soil the results obtained are quite promising.

**Table 5. Soybean cyst nematode population densities and reproductive factor (RF) at Prosper and Crookston before planting the cover crops in the fall 2018, in the spring of 2019 before planting soybean and fall 2019 after harvesting soybean averaged across plots planted to two soybean cultivars [SCN-susceptible (S) and SCN-resistant (R)]**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Fall 18 | | Spring 19 | | Fall 19 | |  |  |
| Cover crop | S | R | S | R | S | R | RF-S | RF-R |
|  | -------------------------------SCN eggs/100 cm3------------------------------ | | | | | |  |  |
|  | Crookston | | | | | |  |  |
| Winter camelina | 850 | 1000 | 850 | 362 | 4912b | 1587a | 3.67 | 0.07 |
| Brown mustard | 1600 | 325 | 925 | 875 | 8412a | 1212a | 3.23 | 0.04 |
| Check | 1225 | 875 | 1075 | 550 | 7387a | 2087a | 1.39 | 0.13 |
| Mean cultivar | 1225 | 721 | 950 | 596 | 6904a | 1629b | 2.76a | 0.08b |
|  | Prosper | | | | | |  |  |
| Winter camelina | 2887 | 2612 | 2675 | 1800 | 8762a | 212a | 23.7 | 4.1 |
| Brown mustard | 2175 | 2237 | 3012 | 2000 | 3900b | 100a | 67.4 | 5.0 |
| Check | 3187 | 3787 | 1875 | 2350 | 4200b | 1037a | 40.2 | 7.3 |
| Mean cultivar | 2750 | 2879 | 2520 | 2050 | 5621a | 450b | 46.7a | 5.9b |

Different small case letters indicate differences between cultivars at 0.05 significance.

Soybean seed yield was lower in the SCN-susceptible cultivar compared with the SCN-resistant cultivar, averaged across all cover crop treatments in Prosper (Table 6). In Crookston, the SCN-susceptible cultivar had lower seed yield than the SCN-resistant cultivar but the difference was not significant. No differences in seed yield between the check and the plots that had cover crops were observed.

Crude protein content did not differ between both cultivars and cover crop treatments while oil content was slightly higher in the susceptible cultivars averaged across treatments (Table 6).

**Table 6. Seed yield, crude protein and oil content of SCN-resistant (R) and SCN-susceptible (S) soybean cultivars at prosper, ND and Crookston, ND.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Seed yield (bu/acre) | | Protein content (%) | | Oil content (%) | |
| Cover crop | S | R | S | R | S | R |
|  | Prosper | | | | | |
| Winter camelina | 35.4 | 41.9 | 32.3 | 32.2 | 18.5 | 18.1 |
| Brown mustard | 35.2 | 42.2 | 32.5 | 31.9 | 18.3 | 18.2 |
| Check | 35.3 | 43.2 | 32.3 | 32.5 | 18.5 | 17.9 |
| LSD(0.05) | 6.3 | | NS | | 0.3 | |
| Mean cultivar | 35.3b | 42.4a | 32.3 | 32.2 | 18.4 | 18.1 |
|  | Crookston | | | | | |
| Winter camelina | 34.4 | 33.5 | . | . | . | . |
| Brown mustard | 29.3 | 44.0 | . | . | . | . |
| Check | 26.6 | 42.1 | . | . | . |  |
| LSD (0.05) | NS | | . | . | . | . |
| Mean cultivar | 30.1a | 39.9a | . | . | . |  |

***Conclusions***

Fall-seeded or interseeded cover crops were not able to decrease SCN reproduction at any location in the SCN-susceptible cultivar. However, in the SCN-resistant cultivar brown mustard and winter camelina had a lower RF than the control at more than one location in both experiments indicating that these cover crops complement the SCN-resistant soybean to keep SCN population from increasing. SCN-resistant soybean suppressed SCN reproduction across all treatments and locations. Interseeding cover crops did not reduce soybean yield, except for the non-terminated brown mustard in Casselton. Oil and protein content were not affected by cover crops treatment, but by soybean cultivar at some locations. The SCN-susceptible cultivar did have lower yield.

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